REMARKS

Reconsideration of the application in view of the above amendments and the following remarks is respectfully requested.

Claims 9, 11 and 12 are pending in the subject application. In order to increase the clarity of the claimed invention, claim 9 has been amended to delete the term "essentially" from the phrase "consisting essentially of". Support for the amendment is found, for example, at page 9, lines 8-11, of the subject application. No new matter has been added by the amendment. Therefore, amended claim 9 and claims 11-12 are pending in the subject application.

In the Office Action dated August 26, 2004, the rejection of claims 9, 11 and 12 under 35 U.S.C. § 103(a) as unpatentable over Carson et al. (U.S. Patent No. 5,679,647) in view of Laus et al. (U.S. Patent No. 6,080,409) was maintained. However, in certain aspects, the Examiner's justification for the rejection has presented new arguments in view of Applicants' prior response. This rejection is respectfully traversed.

As a preliminary matter, the issue of whether the language "consisting essentially of" in claim 9 should be interpreted as "comprising" has been rendered moot. As set forth above, claim 9 has been amended to delete the term "essentially" from the phrase "consisting essentially of". The phrase is now recited in amended claim 9 as "consisting of".

Applicants respectfully submit that the Office Action (at page 4) has misinterpreted Mamula et al. (J. Immunol. 152:1453-1461, 1994) which has resulted in a mischaracterization of Applicants' invention and thus a misapplication of Carson et al. to reject the claimed subject matter. As described in Applicants' prior response (dated June 1, 2004), based on Mamula et al., breaking T cell tolerance to self antigen requires co-immunization with foreign antigens and self antigens. Further, Carson et al. provides no evidence that a foreign protein alone can activate an autoimmune T cell response to a tumor self antigen. Thus, neither Mamula et al. nor Carson et al. teaches Applicants' invention that a foreign protein alone can activate an autoimmune response to a tumor self antigen. In response, the Office Action (at page 4) acknowledges that Mamula et al. teaches co-immunization with foreign and self antigen. However, Mamula et al. is distinguished in the Office Action from Applicants' presently claimed invention with PAP by stating at page 4 that: "In this [Mamula et al.'s] experimental system, the

immune system within the mouse was not subjected to the self-snRNP until the co-immunization because snRNPs are intracellular proteins in normal mice." Applicants agree that snRNPs are intracellular proteins in normal mice. However, as explained below, Applicants respectfully disagree that the fact that snRNPs are intracellular proteins necessarily means that the immune system within the mouse was not subjected to the self-snRNP until the co-immunization.

The assertion in the Office Action, that because snRNPs are intracellular proteins the immune system is not subjected to self-snRNP, is based on the assumption that immune systems are not subjected to intracellular proteins. This assumption is not correct. Whether a protein is or is not an intracellular protein is not determinant as to whether an immune system will be subjected to the protein. An intracellular protein is degraded intracellularly, where it is broken down (processed) into fragments (peptides). Once the protein is processed, the peptides may be bound by class I MHC (major histocompatibility complex) molecules. (It is noted that MHC molecules bind peptides and not intact proteins.) If so bound, the complexes of a peptide and a MHC molecule are then transported from the cell interior to the cell surface for display to cytotoxic T cells. This process of how T cells see intracellular proteins is well known (e.g., Grey et al., Scientific American, November 1989, pages 56-64, particularly pages 58-59; copy of article enclosed for the Examiner's convenience). Thus, the assumption that immune systems are not subjected to any intracellular proteins is not correct. Accordingly, the fact that snRNPs are intracellular proteins does not necessarily mean that the immune system of a mouse in the experimental system described in Mamula et al. was not subjected to the self-snRNP until the co-immunization with human and mouse snRNPs.

Conversely, the assertion in the Office Action, that because PAP is not an intracellular protein the immune system would be subjected to the self PAP, is based on the assumption that the immune system must be subjected to all non-intracellular proteins. This assumption is also not correct. Whether a protein is or is not an intracellular protein is not determinant as to whether an immune system will be subjected to the protein. A non-intracellular protein will be processed into peptides, but at least one such peptide must be bound by a MHC molecule in order to initiate the process of subjecting an immune system to the protein. Even if the level of a protein is elevated, this does not alter the requirement that a

peptide fragment of the protein be bound by a MHC molecule in order for an immune system to be subjected to the protein. (Further, Applicants note that that the standard therapy for prostate cancer is removal of the prostate gland, which is the major source for PAP. Following prostatectomy, the PAP falls to levels substantially below normal.) The Patent Office has presented no evidence to support the assertion: "In the case of PAP, the immune system of patients having prostate carcinoma would be subjected to the self PAP." Accordingly, there is no basis for the conclusion in the Office Action that it would not be necessary to co-immunize with the self PAP protein and the foreign PAP protein.

Therefore, in the Office Action, the misinterpretation of Mamula et al. (i.e., that Mamula et al. teaches that co-immunization is only required because snRNPs are intracellular proteins) has resulted in a mischaracterization of Applicants' invention (i.e., that Applicants' invention of administration of foreign PAP alone is actually co-immunization because PAP is not an intracellular protein and thus immune systems would already be subjected to self PAP). This has led in the Office Action to the misapplication of Carson et al., which only reasonably teaches or suggests co-immunization, to reject the claimed subject matter.

Independent of the fact that human prostatic acid phosphatase (PAP) is not disclosed in either Mamula et al. or Carson et al., neither of these references teaches that a foreign protein alone can activate an autoimmune T cell response to a tumor self antigen. Applicants respectfully submit that Carson et al. is missing more than simply that it does not teach PAP as a tumor associated antigen. There is no reasonable expectation for success of Applicants' method based on Carson et al.

Given that Laus et al. describes a method of stimulating T cell responses to PAP by combining PAP with GM-CSF, it is not clear why there was motivation for one of ordinary skill in the art at the time of Applicants' invention to combine Laus et al. and Carson et al. Nevertheless, assuming motivation in fact existed at that time, the combination of Carson et al. and Laus et al. is at most the substitution of PAP from Laus et al. into the co-immunization method of Carson et al. (It is noted that in Applicants' prior two responses, dated June 1, 2004 and September 26, 2003, Applicants have described in detail why Carson et al. teaches only a co-immunization method.) However, Applicants' presently claimed invention is not a

co-immunization method. Thus, even assuming that it is proper under Section 103(a) to combine Laus et al. and Carson et al., the combination nevertheless still does not teach or suggest Applicants' claimed invention.

Applicants note that Laus et al. individually does not teach or suggest the claimed invention. For example, Laus et al. describes the use of native PAP (in combination with GM-CSF), but does not teach or suggest the use of PAP with an amino acid sequence native to a non-human source.

Applicants recognize and appreciate that the Examiner's maintenance of the application of the combination of Carson et al. and Laus et al. to reject the pending claims was in the context of claim 9 reciting a composition "consisting essentially of" which was interpreted in the Office Action as "comprising". Based on that interpretation, claim 9 was viewed by the Examiner to include co-immunization and, thus Carson et al. was deemed to still be relevant. However, as set forth above, claim 9 has now been amended to delete "essentially", thus rendering moot any question as to whether co-immunization is encompassed by the present claims. Accordingly, the proposed substitution in the Office Action of PAP from Laus et al. into the co-immunization method of Carson et al. is not Applicants' presently claimed invention, and further does not even suggest it.

Even if one were to assume that it would have been *prima facie* obvious to one of ordinary skill in the art at the time Applicants' invention was made to insert the polynucleotide encoding PAP into the method taught by Carson et al., there was no reasonable expectation for success of Applicants' claimed method. There was no reasonable expectation for success based on Laus et al. or Carson et al. or their combination.

Therefore, Applicants believe that the rejection under 35 U.S.C. § 103(a) over Carson et al. and Laus et al. has been overcome. Reconsideration and withdrawal of the rejection is respectfully requested.

Therefore, in light of the amendments and remarks set forth above, Applicants believe that the Examiner's rejection has been overcome. Reconsideration of the application and allowance of the pending claims (9, 11 and 12) are respectfully requested. If there is any further matter requiring attention prior to allowance of the subject application, the Examiner is respectfully requested to contact the undersigned attorney (at 206-622-4900) to resolve the matter.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

Respectfully submitted,

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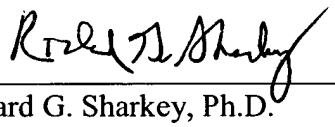

Richard G. Sharkey, Ph.D.
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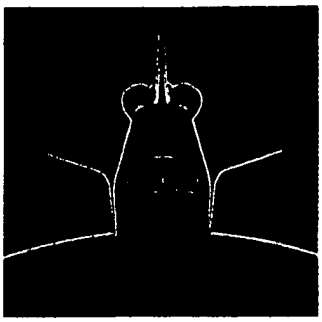
Enclosure:
Grey et al., Scientific American, November 1989, pages 56-64



THE COVER painting is a head-on view of the space shuttle as it flies through the earth's highly reactive uppermost atmosphere (see "Shuttle Glow," by Donald E. Hunton, page 92). The impact of oxygen atoms and nitrogen molecules on the shuttle's surface leads to the formation of excited compounds that emit a diffuse yellow-red glow. Areas without the glow consist of materials that react chemically with the oxygen atoms and are therefore being eroded.

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Scientific American (ISSN 0036-8733), published monthly by Scientific American, Inc., 415 Madison Avenue, New York, N.Y. 10017. Second-class postage paid at New York, N.Y., and at additional mailing offices. Authorized as second-class mail by the Post Office Department, Ottawa, Canada, and for payment of postage in cash. Subscription rates: one year $27, two years $48, three years $66 (outside U.S. and possessions add $11 per year for postage). Subscription inquiries: U.S. only 800-333-1199; other 515-247-7631. Postmaster: Send address changes to Scientific American, Box 3187, Harlan, Iowa 51593.

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PUBLISHER:
John J. Moeling, Jr.

SCIENTIFIC AMERICAN, INC.
415 Madison Avenue
New York, NY 10017
(212) 754-0550

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How T Cells See Antigen

On their own, these key actors in the immune response are blind. Other cells must break down foreign material and enfold it in the body's own proteins before displaying it to the T cells

by Howard M. Grey, Alessandro Sette and Søren Buus

The human body is constantly fighting an imperceptible war against invading microbes and malignant cells. The battle is led by the immune system, which can eliminate or neutralize virtually any invader while sparing the body's own tissues at the same time. The main defenders are the white blood cells called lymphocytes, and the counterattack has at least two prongs. The more familiar one consists of the *B* cells, which react to antigen—distinctively foreign material—by secreting antibodies that bind to the invader. Bolstering the activity of the *B* cells, and supplementing it with a second defensive response, are the *T* cells. These lymphocytes help *B* cells to proliferate and secrete antibodies, and they also kill virus-infected and malignant cells directly.

A precise event triggers the immune response: a receptor molecule on the surface of a *B* cell or a *T* cell encounters the antigen to which the cell is programmed to respond and binds to some small part of it, thereby recognizing it as foreign. Aided by other elements of the immune system, the cell then multiplies and fulfills its role as an antibody-secreting *B* cell, a cytotoxic (cell-killing) *T* cell or a helper *T* cell, which secretes substances that mobilize the other cells. *B* cells perform this feat of recognition on their own, interacting with antigens on bacteria or parasites without any intermediary. Yet isolated *T* cells are blind. What more do they need in order to see a foreign substance?

It has become clear during the past several decades that *T* cells have exacting requirements for recognizing antigen. Another kind of cell must act as a so-called accessory cell, chemically processing the antigen and presenting it to the *T* cell in association with certain of the accessory cell's own surface proteins, known as MHC molecules. Immunologists and molecular biologists are still vigorously probing the intricacies of antigen processing, the nature of the MHC molecules and the role they play in presenting antigen to *T* cells. We have already learned much about this key prelude to the immune response, however. What we know promises to lead to new ways of controlling the immune response. It may aid, for example, in the development of synthetic vaccines and of specific therapies for autoimmune diseases such as multiple sclerosis.

One of the first indications that *B* and *T* cells see antigen in fundamentally different ways came from work done 30 years ago by P.G.H. Gell and Baruj Benacerraf, who were then at New York University. They found that antibodies (and the cells making them) that were specific for a foreign protein in its normal, intricately folded form often ignored it after it had been denatured—disordered or unfolded. Yet the "cell-mediated" immune response, which is the work of *T* cells, was virtually identical for proteins in their normal and denatured forms. *B* and *T* cells were not known at the time, but these experiments suggested in retrospect that *B* cells and the antibodies they secrete must recognize antigen mainly by its shape, whereas *T* cells respond mostly to its makeup—to the sequence of amino acids in the protein chain, which would be identical regardless of how the molecule was folded.

Subsequently, the evidence mounted that *T* cells respond to antigen only when an accessory cell "presents" it. Macrophages, the immune system's scavenger cells, were the first accessory cells to be identified; later, dendritic cells (specialized cells found in the lymph nodes and spleen), *B* cells themselves and, for some kinds of *T* cell reactions, any nucleated cell in the body were added to the list. It turned out that the activity of accessory cells, or antigen-presenting cells (APC's), explains why *T* cells have no interest in antigen shape: the APC's break down the antigen before presenting it, obscuring its shape and leaving only its distinctive amino acid sequence.

Several studies showed that APC's do more than simply capture antigen and display it on their surface. A technique introduced in 1981 by Emil R. Unanue, then at Harvard University, yielded the most compelling results. He and his colleagues exposed APC's

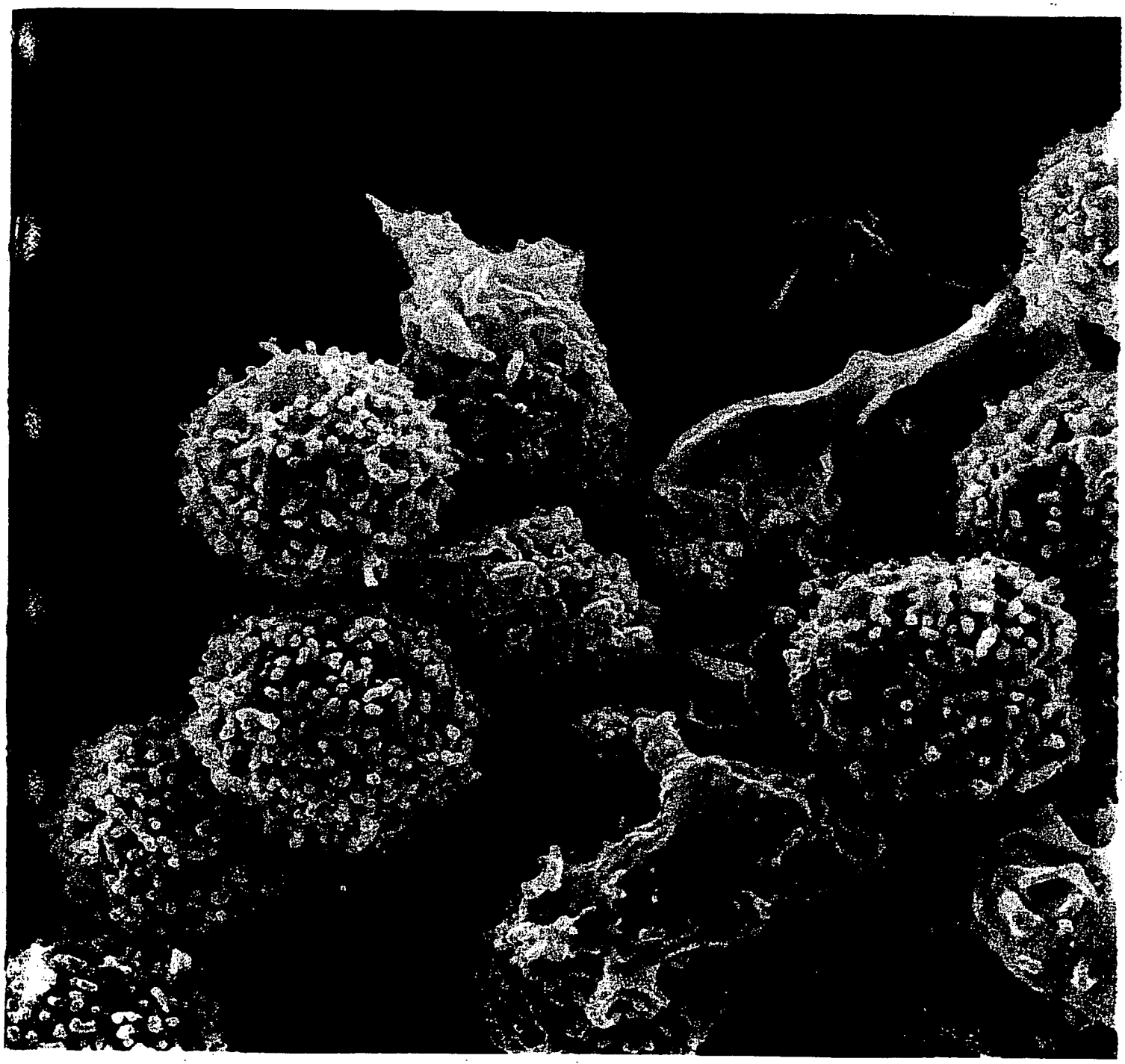
HOWARD M. GREY, ALESSANDRO SETTE and SØREN BUUS have been longtime collaborators in the study of antigen processing and presentation. Grey is co-founder and chief technical officer of the Cytel Corporation in La Jolla, Calif., a biotechnology company that is designing immune-modulating drugs. He got an M.D. from New York University in 1957 and has done research at the Scripps Clinic and Research Foundation in La Jolla and at the National Jewish Center for Immunology and Respiratory Medicine in Denver, where until last year he was head of the division of basic immunology. Sette is senior staff scientist at Cytel and assistant professor of immunology at Scripps. He got his Ph.D. from the University of Rome in 1984 and joined Grey at the National Jewish Center in 1986. Buus, who earned an M.D. at the University of Arhus in 1981, is assistant professor at the Institute of Experimental Immunology at the University of Copenhagen.

T CELLS RECOGNIZE ANTIGEN on the surface of a macrophage, a scavenger cell. The flat macrophage has ingested a bacterial protein, broken it down and displayed the pieces together with certain of the cell's surface proteins. The small, spherical *T* cells are programmed to recognize the bacterial antigens but can only do so with the macrophage's assistance. The scanning electron micrograph is by Morten H. Nielsen and Ole Werdelin of the University of Copenhagen.

to antigen and then, after varying intervals, "fixed" the cells with formaldehyde, which interrupted their metabolism. The workers then tested the cells' ability to present antigen to *T* cells and trigger their proliferation. APC's fixed before or immediately after they were exposed to antigen could not present it to *T* cells. In contrast, APC's that were incubated with antigen for an hour or more and then fixed were perfectly capable of doing so. These results and others suggested that, after exposure to antigen, accessory cells required time and energy before they could present antigen to *T* cells, probably because they had to modify it somehow first.

Other experiments shed light on this process by showing that certain weak bases strongly inhibit the ability of APC's to present antigen. The compounds are probably active in the endosomes, acidic compartments within the cell where ingested material is broken down by proteolytic, or protein-cleaving, enzymes. Presumably by neutralizing the endosomes, the bases inhibit a cell's ability to degrade proteins. Later work showed that specific inhibitors of proteolytic enzymes also interfere with antigen presentation.

The possibility that cleavage of antigen into short fragments, or peptides, prepares it for presentation to *T* cells gained crucial support from an experiment done by Richard P. Shimonkevitz, Philippa C. Marrack and John W. Kappler, all of the National Jewish Center for Immunology and Respiratory Medicine in Denver, and one of us (Grey). The group showed that single peptides derived from a protein antigen could substitute for the intact protein in triggering a *T* cell response. The peptides clearly needed no further processing to do so, since they could be presented by APC's that had



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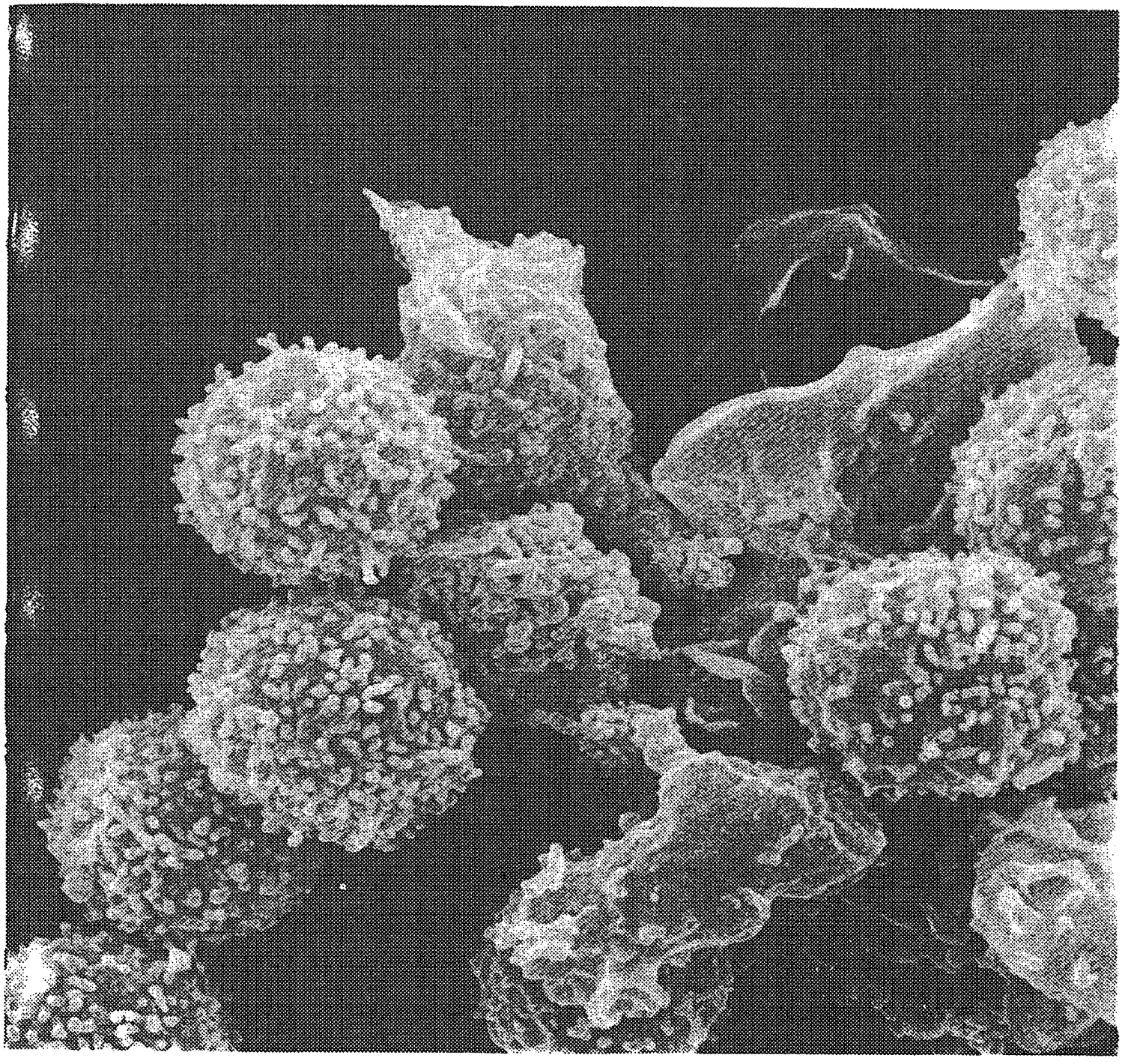
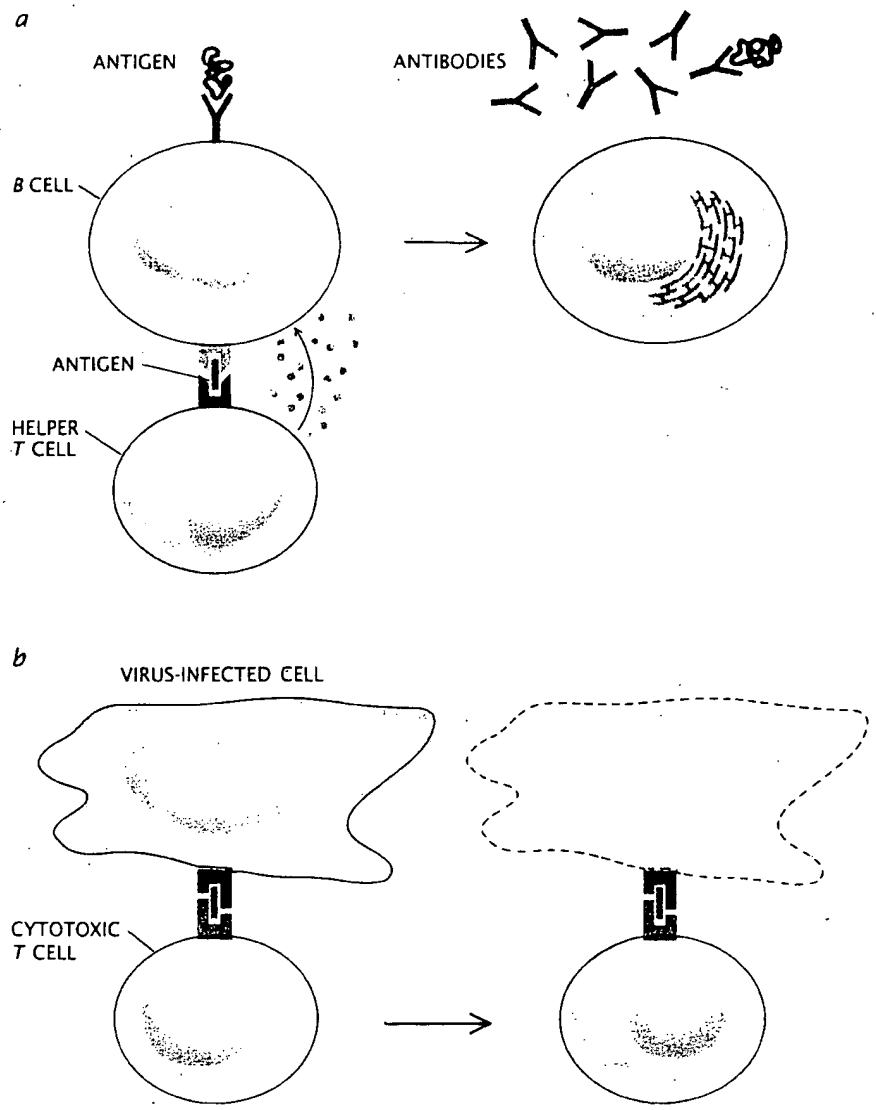




T CELLS HAVE A DUAL ROLE. A helper *T* cell recognizes antigen (*color*) on the surface of another cell (in this case a *B* cell) that has encountered foreign material and broken it down (*a*). The *T* cell responds by secreting substances that help other immune-system cells to fulfill their roles. (Here it stimulates the *B* cell to mature and secrete antibodies.) A cytotoxic, or killer, *T* cell recognizes antigen on the surface of a virus-infected or malignant cell and responds by killing the target cell (*b*).

previously been fixed. Recently Stephane O. Demotz in our laboratory at Cytel actually isolated a processed antigen and determined that it is indeed a short peptide. In one account of antigen processing, then, an APC engulfs antigen and delivers it to acidic compartments within the cell, where it is broken down into small peptides, as short as 10 to 20 amino acids, before being returned to the cell membrane for recognition.

That is only a partial account of antigen processing, however. The steps it describes occur in the specific classes of antigen-presenting cells—*B* cells, macrophages and dendritic cells—that are specialized for processing foreign material taken in from the surrounding medium. The processing and presentation of such "exogenous" antigens generally leads to the activation of a specific population of *T* cells: the helper cells that aid *B* cells in making antibody.

Not all the antigens recognized by *T* cells originate outside the presenting cells, however. A cell that has been infected by a virus or has become malignant may synthesize distinctive, virus- or tumor-specific proteins. Virtually all cells in the body can present such internally synthesized proteins, and they do so to *T* cells belonging to the second major population: the cytotoxic *T* cells. These lymphocytes respond to "endogenous" antigens by killing the cells that produce them.

Until recently many workers assumed that such endogenous antigens did not need to be processed, since the intact proteins are often expressed on the surface of the abnormal cells. It seemed plausible that cytotoxic *T* cells, unlike helper cells, might be able to respond directly to intact antigen. Yet Alain Townsend of the John Radcliffe Hospital in Oxford found in 1985 that cytotoxic cells capable of killing cells infected with a virus could also kill uninfected cells into which a mere fragment of a viral gene had been introduced. These genetically engineered cells produced only a small fraction of the corresponding viral protein found on the infected cells. The cytotoxic cells nonetheless responded identically to both molecules. Later Townsend induced a cytotoxic response with uninfected APC's that had merely been incubated with a short antigenic peptide, confirming that cytotoxic cells, like helper cells, recognize a fragment of antigen and not the complete protein.

Other experiments indicated, however, that infected cells process endogenous antigens by a mechanism quite different from the one that prepares antigen for recognition by helper cells. The weak bases that blocked processing for helper *T* cell recognition and pointed to a central role for endosomes in that processing pathway had no effect on antigen presentation to cytotoxic *T* cells. Moreover, when an antigenic protein was added to a culture containing APC's and antigen-specific cytotoxic *T* cells, nothing happened. The killer cells were able to recognize and respond to the protein, however, when it was microinjected into the cytoplasm—the fluid interior medium—of the presenting cells.

The data are not yet completely definitive, but they are most compatible with a picture in which endogenous antigen is processed in the cytoplasm rather than within endosomes. Once the protein has been degraded in the cytoplasm, the fragments are somehow moved into the interior of a vesicle, a sac that shuttles between the cell interior and its surface. The peptides are then transported to the cell surface for recognition by killer *T* cells.

This second processing pathway, specialized for antigens made by the APC itself, could be the immune system's way of ensuring that a foreign organism cannot elude it by adopting a Trojan-horse strategy. Even if the pathogen is hidden within a cell, the body will process the novel proteins and make them visible to the *T* cells.

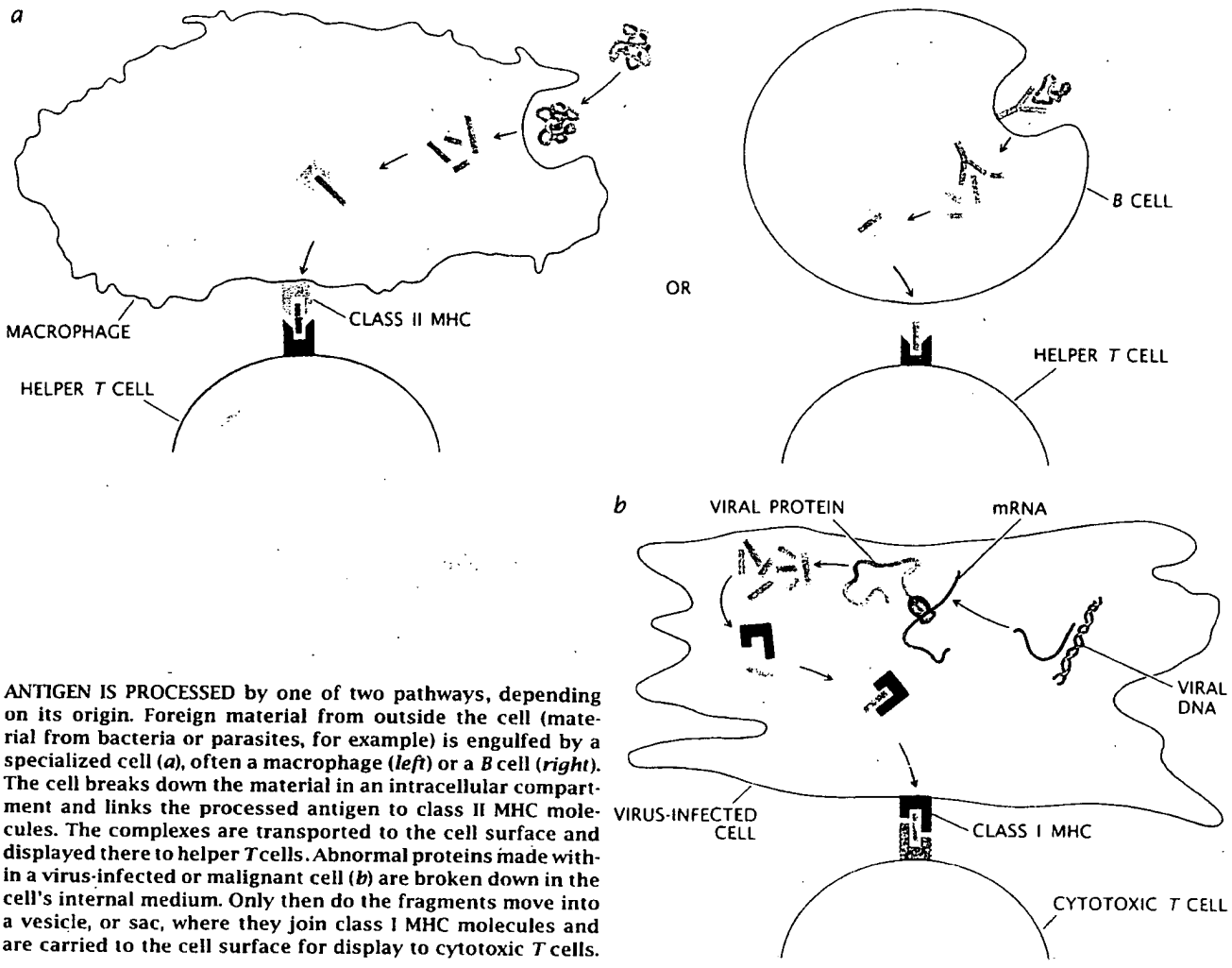
In addition, the existence of two separate pathways of antigen processing, one for exogenous antigens and one for endogenous antigens, makes biological sense: each pathway leads to the appropriate *T* cell response. A bacterial protein taken up by a *B* cell from its surroundings and processed by the exogenous pathway elicits *T* cell help, which enables the *B* cell to produce antibodies for combating the infection. A foreign or abnormal protein made by a renegade cell, in contrast, leads to the killing of the errant cell by cytotoxic *T* cells.

Once it has been processed, antigen is displayed on the surface of the accessory cell together with proteins of the cell's own making. They are known as MHC proteins, after the major histocompatibility gene complex, a cluster of more than a dozen genes. The cluster is a hot spot of genetic variability, so that the MHC proteins encoded by a given set of genes almost always differ from one individual to the next. The molecules do fall into two broad classes, however, according to their structure and their role in *T* cell stimulation. Class II MHC proteins, found mainly on the surface of *B* cells, macrophages and dendritic cells, figure in the presentation of antigen to helper *T* cells. Class I proteins, found on almost all nucleated cells in the body, play the same role for cytotoxic *T* cells.

The current picture of MHC molecules and their part in stimulating the *T* cell response is the product of more than three decades of investigation, beginning in the mid-1950's with studies of tissue grafts. Investigators found that when tissue from one animal was transferred to another one with different MHC proteins, the immune system of the recipient rejected the graft in an extraordinarily intense reaction, one that was later traced to *T* cells. It appeared that the immune system, and its *T* cell arm in particular, is "tuned" to recognizing MHC molecules. Clearly, though, their normal immunologic function had to be something other than graft rejection. After all, grafts are rare in nature.

An early hint of a normal function for MHC proteins came from experiments done in the 1960's by Hugh O. McDevitt, who was then at the National Institute for Medical Research in England, and Benacerraf. They showed that the genes of the MHC affected an animal's ability to mount an immune response to certain simple antigens. An animal carrying one variant of a particular MHC gene might respond vigorously to a given antigen; another animal carrying a different variant might not respond at all. In these responder and nonresponder strains, the MHC seemed to function as "immune response" genes.

How might these genes affect the immune response? The most obvious explanation was that they encoded the *T* cells' own receptor molecules. In 1973, however, Alan S. Rosenthal and Ethan M. Shevach of the National Institute of Allergy and Infectious Diseases made an observation that linked the MHC to the function of the accessory cells. They mated a guinea-pig strain



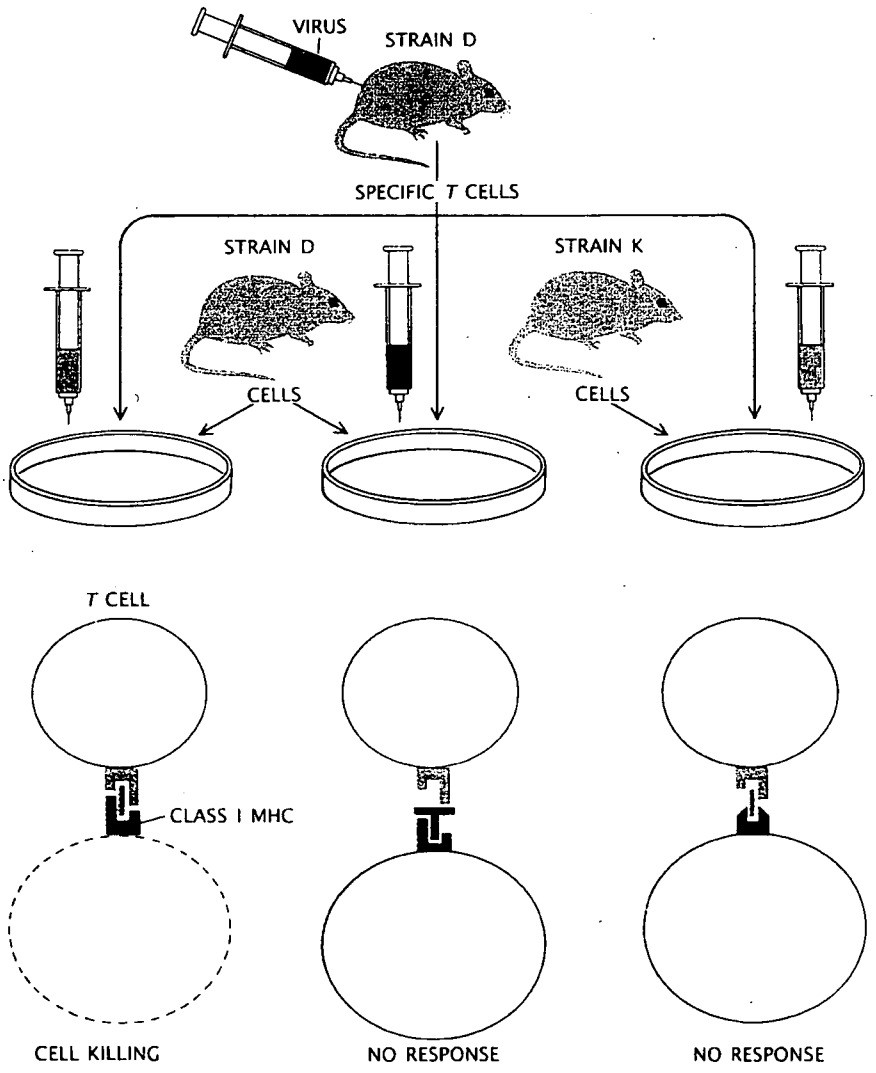
ANTIGEN IS PROCESSED by one of two pathways, depending on its origin. Foreign material from outside the cell (material from bacteria or parasites, for example) is engulfed by a specialized cell (*a*), often a macrophage (*left*) or a *B* cell (*right*). The cell breaks down the material in an intracellular compartment and links the processed antigen to class II MHC molecules. The complexes are transported to the cell surface and displayed there to helper *T* cells. Abnormal proteins made within a virus-infected or malignant cell (*b*) are broken down in the cell's internal medium. Only then do the fragments move into a vesicle, or sac, where they join class I MHC molecules and are carried to the cell surface for display to cytotoxic *T* cells.

that responded well to one antigen and poorly to a second antigen with a strain that showed the reciprocal pattern, responding poorly to the first antigen and well to the second. The offspring—having inherited a gene for responsiveness from each parent—could mount a strong response to both antigens. But when the workers extracted *T* cells from the hybrid animals and mixed them with APC's and antigen in culture, the helper *T* cell response depended on the origin of the accessory cells.

In the presence of APC's that had also come from the hybrid animals, the *T* cells responded to both antigens, as expected. When the APC's had been isolated from the parental strains, however, the *T* cells reacted only to the antigen to which the parental strain had also responded. The cells seemed blind to the other antigen. It appeared that the MHC genes exerted their effect not through the *T* cells themselves (under the right conditions the *T* cells of the hybrid animals were perfectly capable of responding to both antigens) but through the APC's. Somehow accessory cells carrying the nonresponder genes were unable to present one of the antigens—a phenomenon for which the mechanism has only recently been elucidated.

Meanwhile studies of cytotoxic *T* cells led to the conclusion that *T* cells recognize not only foreign antigen but also the MHC-encoded proteins on the accessory cells. In 1974, for example, Rolf M. Zinkernagel and Peter C. Doherty of the Australian National University exposed *T* cells that had responded to antigen presented by cells carrying a particular variant of class I MHC protein to the same antigen presented by cells bearing a different MHC variant. First, the workers infected a mouse with a virus, stimulating cytotoxic *T* cells targeted to the virus-infected cells. Then they extracted the specific *T* cells and exposed them in vitro to virus-infected cells from other mice.

Zinkernagel and Doherty found that if the class I MHC proteins on the surface of these new infected cells differed from those of the original mouse, the cells escaped *T* cell killing. The workers interpreted the results as showing that an animal's *T* cells had to recognize two entities in order to respond: both an antigen and a specific MHC protein—one that is characteristic of the animal's own cells. Confirmed by many other experiments, this requirement for co-recognition of antigen and a "self" MHC molecule became known as MHC restriction.



MHC RESTRICTION of the *T* cell response, discovered by Rolf M. Zinkernagel and Peter C. Doherty of the Australian National University in 1974, consists in *T* cells' need to recognize both a specific antigen and a specific MHC protein. The workers infected mice of a specific MHC strain with a virus and isolated virus-specific cytotoxic *T* cells. In culture, these lymphocytes could kill cells from uninfected mice of the same strain when the cells were infected with the same virus (*left dish*) but not when the virus was different (*center dish*). They also failed to kill cells from another strain carrying the same virus but bearing a different MHC protein (*right dish*).

This MHC restriction of the *T* cell response presented a new puzzle. *B* cells, after all, are activated by the fit of a single key (the antigen) into a single lock (the receptor on the *B* cell surface). What might be the molecular design of the *T* cell's double-key system? One theory held that *T* cells bear two independent receptor molecules, one specific for antigen and the other for a self-MHC protein. A second theory postulated that *T* cells carry a single receptor molecule capable of identifying both antigen and MHC. Proponents of each theory raised indirect evidence in its favor; the controversy was settled in favor of the one-receptor model when a single *T* cell receptor was shown to be specific for both antigen and self-MHC [see "The *T* Cell and Its Receptor," by Philippa Marrack and John Kappler; SCIENTIFIC AMERICAN, February, 1986].

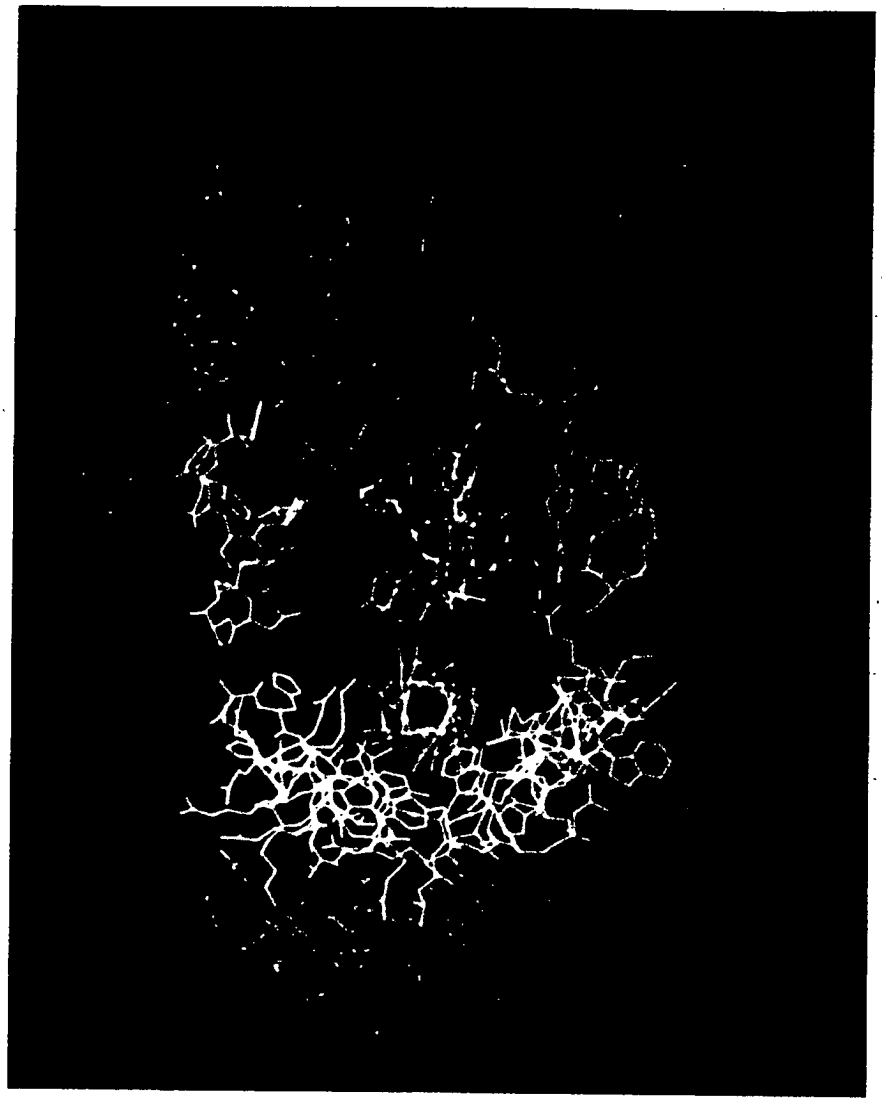
The existence of a single receptor suggested that the processed antigen and the MHC molecule might form a complex—a single entity that would fit a single recognition site in the *T* cell receptor. In effect, the MHC protein would act as the primary receptor for processed antigen; the resulting complex would then interact with a second receptor, on the *T* cell. Because both the antigen and the MHC would contribute to shaping the molecular char-

acteristics of the complex, the proposed mechanism would elegantly explain *T* cells' specificity for both MHC and antigen. It might also explain the puzzle early studies had posed: How do certain MHC genes render an individual blind to specific antigens? In this new picture, those genes might encode proteins unable to bind and present particular peptides.

Ronald H. Schwartz of the National Institute of Allergy and Infectious Diseases provided compelling but indirect evidence in favor of complex formation. He studied the ability of mice belonging to different MHC strains to react to variants of a particular protein. He found that whereas a specific variant might elicit a *T* cell response in one strain but not in a second one, a difference of a few amino acids in the protein's sequence might make it visible to the immune system of the second strain. Schwartz argued that such results were best explained by supposing that the protein—or a peptide cleaved from it—had to bind to MHC molecules before it could trigger a response. The slight difference in amino acid sequence was what was needed for the peptide to bind to the MHC molecules of the second strain.

In 1985 Unanue and his colleagues at Washington University were the first to demonstrate complex formation directly, by means of a technique called equilibrium dialysis. A chamber containing an antigenic peptide was separated by a semipermeable membrane from another chamber containing the class II MHC molecule that restricted the immune response to the antigen. The antigen—by far the smaller molecule—could pass through the membrane, but the MHC protein was confined on one side. All else being equal, the antigen should have diffused through the membrane until its concentration in both chambers was the same. Instead its concentration grew larger on the side that also contained the MHC protein. Evidently, the molecules were binding to each other.

Our group demonstrated the same kind of interaction for a variety of peptides and class II MHC molecules. We also showed that the binding is critical for an immune response: *T* cells recognize complexes of MHC and antigen. Adopting a technique developed in Harden M. McConnell's laboratory at Stanford University, we embedded antigen-MHC complexes into an artificial lipid membrane—a simulated cell membrane. For comparison, we also prepared membrane containing uncomplexed MHC, bathed in free antigen. The preformed complexes stimulated antigen-specific *T* cells some 20,000 times more efficiently than did the uncomplexed MHC and antigen.



CO-RECOGNITION of antigen and an MHC protein is modeled. A *T* cell's receptor approaches from above and encounters a foreign peptide (*pink pinwheel*) in the antigen-binding cleft (*yellow trough*) of the MHC protein. *T* cell receptors include fairly constant regions (*yellow*), which interact with the body's small range of MHC proteins, and a highly variable region (*pink*), for recognizing diverse antigens. The computer model is by Mark M. Davis and Pamela J. Bjorkman of Stanford University.

For such complexes to have a role in the normal immune response, they must be quite stable: in any individual only a few *T* cells bear receptors specific for a given antigen, so that after an individual's exposure to the antigen it may take some time before a specific *T* cell encounters an APC bearing the antigen-MHC complex. The success of our experiment suggested that the complexes are indeed stable, since it took us more than a day to isolate antigen-MHC complexes and embed them in the lipid membrane. Direct measurements of the complexes' dissociation rates confirmed their stability: at body temperature their half-life was about 10 hours.

Compelling as the evidence of complex formation was, not everyone accepted the further proposal that a failure of some MHC proteins to bind certain antigens underlies the genetic unresponsiveness investigators such as Rosenthal and Shevach had studied. Experiments with different antigens did not always support Rosenthal and Shevach's conclusion that such immunologic blind spots reflect a deficit in the antigen-presenting cells. Also, some workers pointed out that it was hard to see how a single MHC protein could act as a specific receptor for myriad struc-

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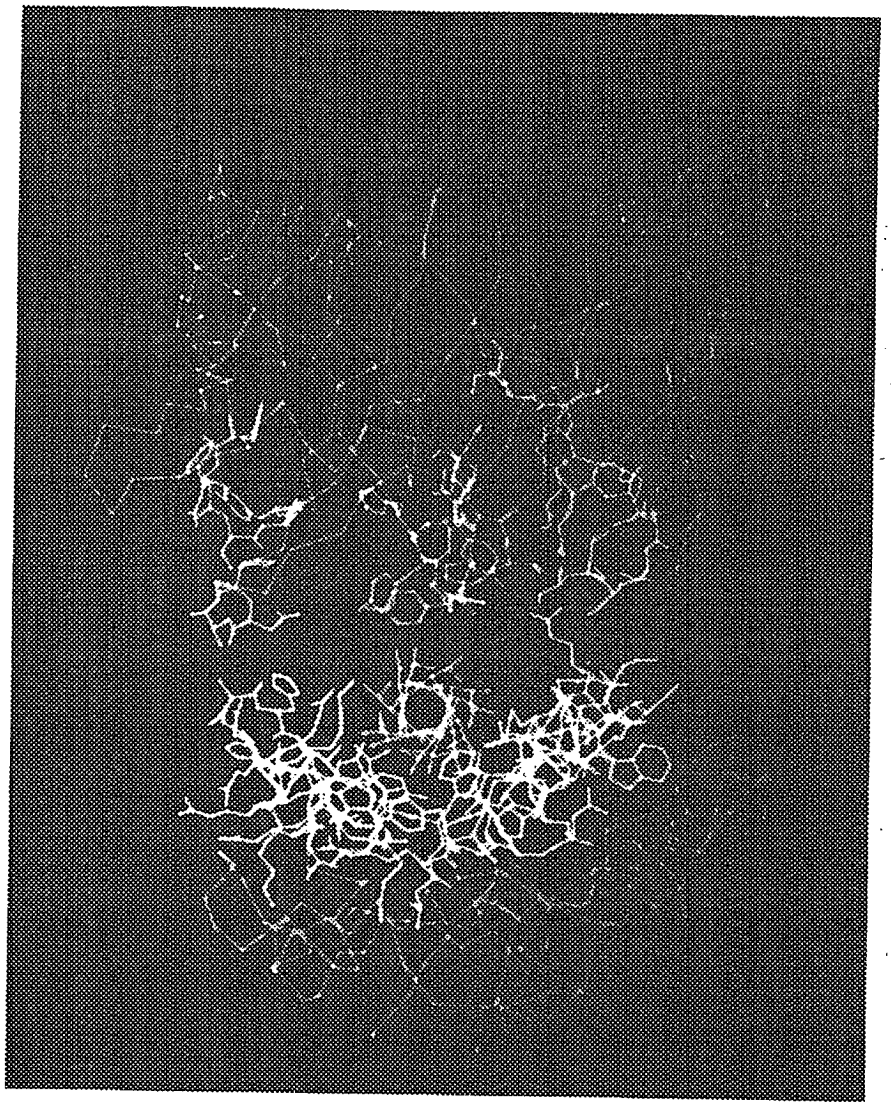

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turally distinct antigenic peptides.

After all, each individual has at most about a dozen MHC proteins. How could the MHC proteins be selective when each one must bind a sizable part of a vast universe of potential antigens? In this view, antigen-MHC complexes, if they existed, had to form nonspecifically. Variations in immune responsiveness had to reflect something other than selective binding.

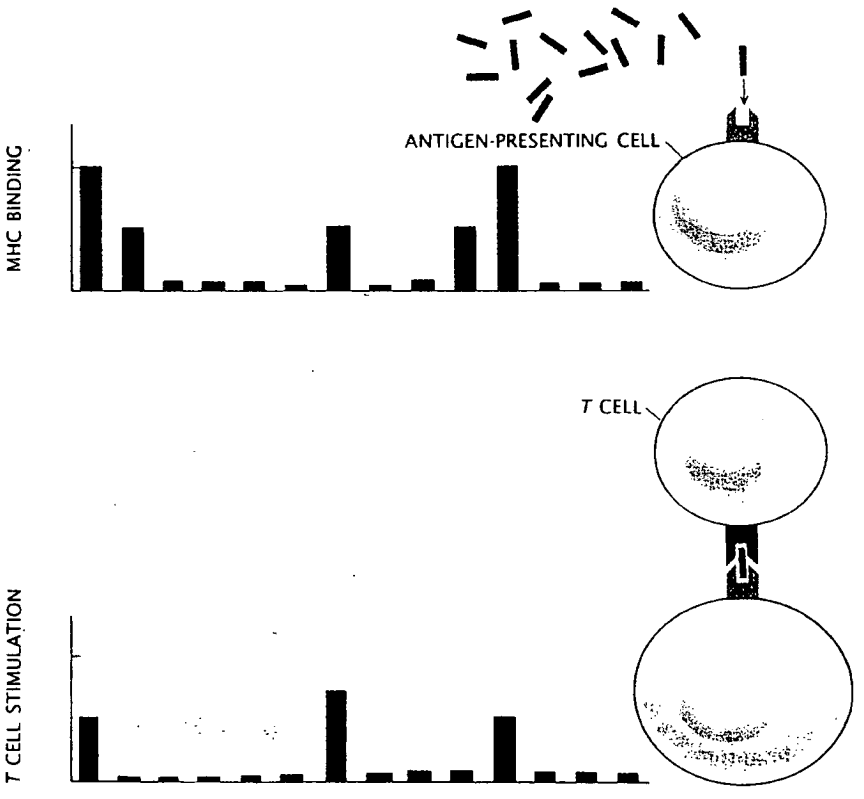
Some investigators proposed instead that the MHC influences the immune response by shaping the repertoire of functional *T* cells. *T* cells mature in the thymus gland, and in the process they interact with the MHC proteins on the surface of accessory cells in the thymus. During this thymic "education," the *T* cells learn to recognize antigen only in association with the body's own MHC molecules. At the same time, it is thought, *T* cells that bind too avidly to self-MHC—and hence pose the threat of an autoimmune reaction—are eliminated or at least inactivated. Conceivably, a particular variant of a self-MHC protein might lead to the elimination of all the *T* cells capable of reacting to a particular antigen. Any individual inheriting the corresponding MHC gene would display the same hole in the *T* cell repertoire.

We tested the relative influences on immune responsiveness of MHC binding and holes in the *T* cell repertoire by comparing peptides' ability to bind to a mouse MHC molecule with their ability to induce an immune response. Of a set of 14 peptides—which in sum represented an entire protein molecule—five could bind to the MHC protein; three of those five, we found, could then trigger a *T* cell response in animals of the same MHC strain. None of the peptides that failed to bind could stimulate a response.

The selectivity of the MHC proteins, then, does shape the immune response. But not every peptide that can bind to a self-MHC elicits a response; some antigens that bind fail to stimulate a response, apparently because *T* cells able to recognize the antigen-MHC complex are absent. Both theories of how the MHC genes influence the immune response appear to be correct. The selectivity of MHC proteins in binding antigens combines with holes in the *T* cell arsenal to set the boundaries of an individual's immune responsiveness.

The earlier objection to the notion of MHC proteins as specific antigen receptors remained unanswered, however: How could an MHC protein selectively bind many—but not all—antigens? We found that a typical MHC molecule can indeed bind between 10 and 20 percent of the peptide fragments from any given protein molecule. We also identified a possible basis for this broad but selective binding: peptides bound by a particular MHC molecule turned out to share certain simple structural features.

One MHC molecule, for example, bound peptides that all shared a motif of repeated hydrophobic residues—amino acids with an affinity for a nonwater medium. Another MHC molecule bound peptides that had in common a trio of positively charged residues. Perhaps such diverse, broad specificities give an individual's array of MHC proteins the ability to bind and present the widest possible variety of antigens, so that a foreign substance is unlikely to slip through the immune system's defenses.



IMPORTANCE OF MHC BINDING to an antigen's ability to stimulate a *T* cell response was assessed by the authors. They synthesized 14 peptides representing fragments of a protein and measured the affinity of each one for a mouse MHC molecule (*top*). Five of the peptides bound to the molecule. Three of the five could stimulate a *T* cell response in a mouse of the same strain (*bottom*). Binding to an MHC protein appears to be necessary, but not sufficient, for a peptide to trigger an immune response.

Vivid confirmation that MHC molecules serve as receptors for processed antigen exported to the cell surface came in 1987, when Don C. Wiley and his colleagues at Harvard University solved the three-dimensional structure of a class I MHC molecule. The most striking feature of the structure, determined from the diffraction pattern of X rays trained on a crystal of the protein, was a cleft on the top of the molecule, where it would face outward from the cell surface. Two helical regions of the protein form the walls of the cleft; so-called beta sheets, in which the protein chain folds back and forth in a plane, form the cleft's floor.

The cleft *looks* like the binding site for an antigenic peptide. What is more, many of the variable amino acids that distinguish a particular MHC protein in different individuals and affect immune responsiveness turn out to be clustered on the inside walls and floor of the cleft. Such amino acids presumably influence the protein's peptide-binding ability; one would therefore expect them to mark the binding site.

A second observation also pointed to the cleft as the peptide-binding site and raised an intriguing new possibility about the function of MHC molecules. The cleft was not empty: in it Wiley and his colleagues identified another molecular entity. The material

must have been bound to the MHC molecules when they were crystallized; presumably, it was a piece of processed antigen.

Paul M. Allen of Washington University and our own group have confirmed that the binding site on MHC proteins of accessory cells is routinely occupied. Acid treatment of class II MHC molecules purified from *B* cells released peptides that later could rebind specifically to the MHC molecules. What is more, Townsend and his co-workers recently showed that a cell cannot even assemble class I MHC molecules properly unless a peptide is present during the final stages of the protein's folding process. It seems likely that these omnipresent peptides are fragments of the body's own proteins, produced within the cell or captured from its surroundings, that have been processed and presented by the same mechanisms that display foreign antigens.

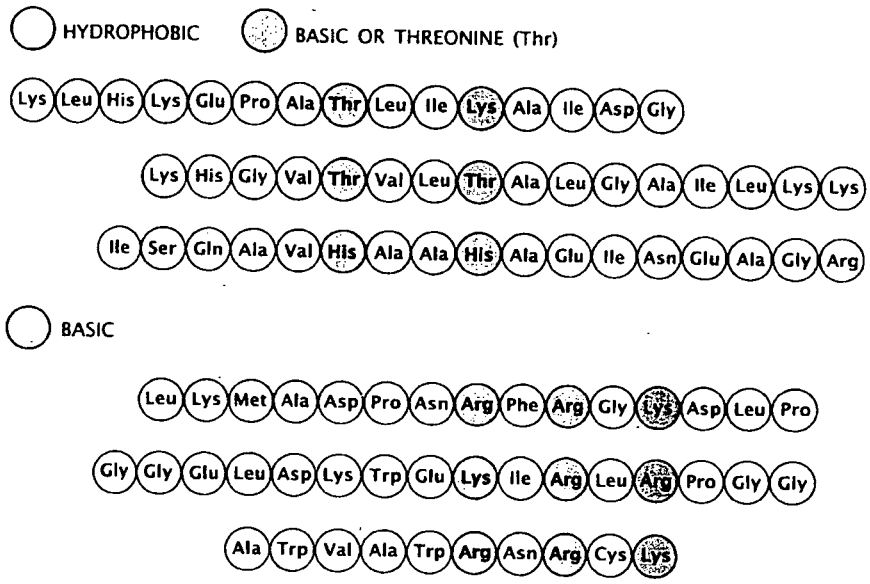
That proposal is consistent with the theory of immune surveillance, which holds that killer *T* cells constantly monitor the other cells of the body for the appearance of tumor or viral antigens and promptly eliminate any cell expressing them. By continuously processing and presenting their own antigens, cells in effect invite inspection by the immune system, so that it can quickly detect any aberration.

This scenario of constant self-scrutiny suggests an answer to the inevitable question about antigen presentation to *T* cells: Why does it need to be so elaborate? Why do *T* cells not recognize antigen directly, as *B* cells do, instead of requiring it to be broken down and displayed in the context of MHC molecules? For cytotoxic *T* cells, one answer is that MHC restriction targets them to the body's own tissue, where—being killer cells—they are designed to act. Because the cells are "interested" in self-MHC as well as antigen, they look for antigen in precisely the setting in which they can respond effectively to it.

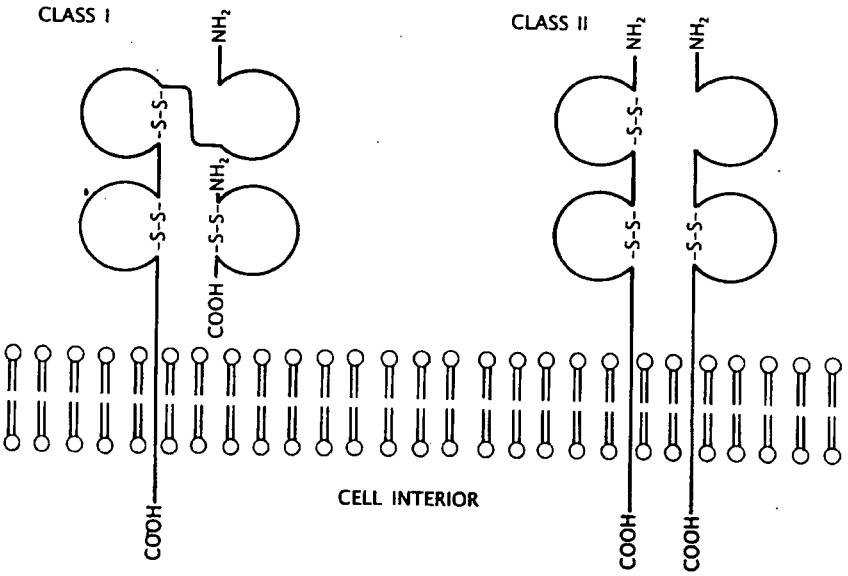
For the equally complex scheme of antigen presentation to helper *T* cells, one might invoke an evolutionary explanation. Cell-mediated immunity appears to be ancient; even organisms as primitive as sponges can recognize and prevent invasion by cells from different species. Thus, *T* cells may have originated as killer cells, but even when they acquired an additional, helper role, they retained a disposition to look for antigen on the cell surface, in association with the body's own proteins. Over the course of evolution, this interest in self-proteins became adapted to the helper cells' function, so that class II MHC proteins now guide these *T* cells to a site where they can be most effective—to *B* cells, the primary target of *T* cell help.

Even though the picture of antigen processing and presentation is far from complete, understanding of the phenomenon has advanced dramatically. The result is likely to be an improved ability to manipulate the immune system for clinical purposes: to stimulate immunity with vaccines and selectively suppress it in autoimmune diseases.

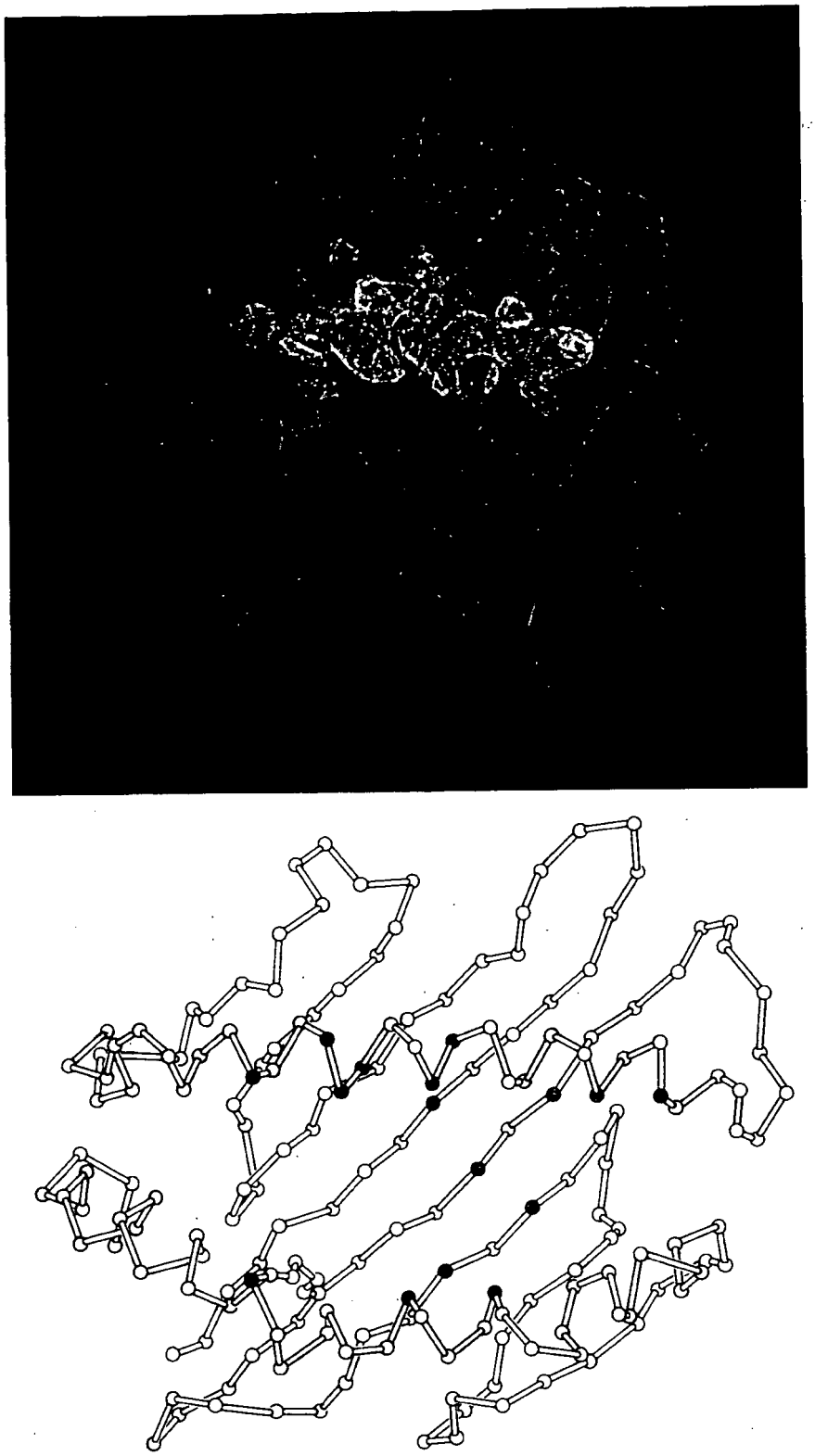
Traditionally, vaccines have consisted of the whole pathogenic organism, live or killed, or a protein extracted from it. For some diseases, such as malaria, that approach is not feasible, and some whole-organism vaccines



DISTINCTIVE STRUCTURAL MOTIFS characterize peptides able to bind to particular MHC proteins. The upper three peptides all bind well to one MHC molecule; the lower three all bind well to another such molecule. Peptides in each cluster share a common pattern defined by the chemical properties of their constituent amino acids.



MHC MOLECULES are divided by structure into class I, found on almost all cells, and class II, found only on specialized antigen-presenting cells. Each protein consists of two chains. In class I molecules one chain extends through the cell membrane and the other lies outside the cell; in class II molecules both chains extend through the membrane. Bonds between sulfur atoms (*S*) divide the chains into looping domains. On proteins of both classes the binding site for antigen lies within the upper loops.



ANTIGEN-BINDING CLEFT of a class I MHC molecule is shown in two images based on X-ray analysis of the protein: a computer model (*top*) and a diagram (*bottom*), where the cleft lies between the two helixes. The discovery of a separate substance (colored orange in the computer image) lodged in the cleft supported the proposal that it is the binding site for antigen. In addition, many of the variable amino acids (shown in red in the diagram) that affect a particular MHC molecule's antigen-binding capability are clustered in the cleft. Don C. Wiley of Harvard University and his colleagues Pamela J. Bjorkman, Mark A. Saper, Boudjema Samraoui, William S. Bennett and Jack L. Strominger determined the molecular structure and provided the computer image.

have risky side effects. In such cases vaccine developers are now trying to design synthetic peptides (representing only a small part of the actual antigen) that will trigger an equivalent immune response. To do so, the peptides must stimulate helper and cytotoxic *T* cells as well as *B* cells, and so it is critical that these antigens bind to MHC molecules in spite of individual variation. The deepening understanding of MHC-antigen interactions will surely help guide the design of such peptide vaccines.

It may also help in treating insulin-dependent diabetes, rheumatoid arthritis and multiple sclerosis—diseases in which the immune system loses its ability to discriminate self from nonself and responds to the body's own molecules. Some of these diseases almost exclusively affect people carrying specific MHC genes. The corresponding MHC proteins may play a role in the diseases by presenting self-antigens in a way that induces an immune response.

MHC-based technology should make it possible to develop compounds that bind very strongly to the disease-associated MHC proteins. By blocking the binding of self-antigens, such compounds might suppress the autoimmune response. It is already possible to arrest some of these diseases by means of an immunosuppressive agent (such as cyclosporine), which blocks the immune response generally. A blocker targeted to a specific MHC variant, however, would have the advantage of leaving the immune system largely intact and able to defend the body against external threats. Growing understanding of antigen processing and presentation may thus give the fight against autoimmune diseases some of the immune system's own precision and power.

FURTHER READING

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ANTIGEN PRESENTATION PATHWAYS TO CLASS I AND CLASS II MHC-RESTRICTED T LYMPHOCYTES. Thomas J. Braciale et al. in *Immunological Reviews*, No. 98, pages 95-114; August, 1987.

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